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ATTRACTION STUDIES WITH THE WESTERN PINE BEETLE

SEASON OF 1929

by

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Assistant Entomologist

Berkeley, Calif.
March 1, 1930

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SUMMARY

Attraction studies have been carried on for the last two years at the North Warners field laboratory as a part, of the larger study of the tree selection habits of the western pine beetle. The purpose of the attraction tests, supplemented by work on the chemistry of the oleoresin and the sugars of the inner bark, is to determine the nature, and the physiology of the formation, of substances which will make certain trees attractive to the beetles.

The 1928 studies indicated chiefly that the western pine beetle is positively chemotropic toward western yellow pine; that sugars of the inner bark or products of their decomposition are more attractive than the essential oils of the bark and wood; that the inner bark is the most attractive part of the tree; and that fermented inner bark is especially attractive to the western pine beetle.

The 1929 tests were planned to check the results of the 1928 studies by more extensive tests, and also to study the change in the attractiveness of a tree after being felled and the possible repellent effect of turpentine and bark oils distilled from the bark of western yellow pine.

The capacity of the testing equipment was enlarged in 1929, and methods were improved so that more tests could be made and so that the influence of such factors as light and temperature were largely eliminated.

As a result of the studies to date, the following more or less tentative conclusions have been reached:

1. The inner, living bark is the most attractive major part of the tree. The outer bark ranks second in attractiveness, the sapwood third, and the needles are the least attractive of the parts of the tree that were tested. (See Chart 1)

2. No consistent difference was found in the attractiveness of slow- and fast-growing trees, as a whole, after being felled.

The outer bark removed from slow-growing trees was found to be more attractive than that of fast-growing trees, probably due to the repellent or neutral effect of the excess of volatile oils found in the outer bark of the fast-growing trees (See Chart 2 and Page 9). On the other hand, inner bark removed from slow-growing trees was evidently a little less attractive than inner bark from fast-growing trees (Chart 2). These results with felled trees are at variance with the results of studies with living trees, which showed a definite preference by the beetles for the slower-growing trees.

3. The attractiveness of a tree increases almost immediately after being felled. The degree of attraction apparently increases for a few days and then decreases.

> The rate of change would vary principaplly with the temperature, but the trees studied were apparently most attractive from three to six days after being felled, after which they decreased in attractiveness until after a week or two they were less attractive than newly-felled trees (Charts 3 and 4 and Plate 2).

4. Fermenting inner bark, if comparatively fresh, is the most attractive of the substances tested.

> In comparative tests with unfermented inner bark, fermenting bark was found to be more attractive for four days after inoculation and less attractive after four days. The attractiveness would vary with the type of yeast or yeasts causing the fermentation and the rate of change would vary with the temperature (Chart 5, Plate 3).

> Inner bark inoculated with a pure yeast culture was found to be consistently nearly twice as attractive as moistened cotton in tests extending over a period of two weeks (Plate 3).

> Other miscellaneous tests indicate considerable variation in the effect of fermentation, depending on the type of inoculation and the age of the fermentation. It was found that pure yeast cultures in suger media were less attractive than either fresh or fermented inner bark, and that fermenting inner bark from fast- and slow-growing trees was equally attractive (Chart 7).

5. Turpentine and bark oils distilled from western yellow pine are apparently neither attractive nor strongly repellent (Chart 8).

> Mirov found that some of these oils were much more repellent than others, and that the lower the boiling point the less attractive the oil.

As a result of all the work that has been done on the tree selection problem, a tentative "theory of attraction" has been developed. According to this theory the beetles are attracted to certain trees by odors from highly volatile substances, such as aldehydes and esters, which are produced as by-products of respiratory fermentation in the inner bark of subnormal trees. The principal value of this theory is that it will help to give direction to future work which should be directed toward proving or disproving this theory.

Because of the variability in the response of the different beetles, it seems highly improbable that any substance will be found that is sufficiently attractive to be of value as a control measure.

ATTRACTION STUDIES WITH THE WESTERN PINE BEETLE
SEASON OF 1929

Introduction

Studies on the source of the attraction that results in the selection by the western pine beetle of certain trees were begun in 1927 and continued through 1928 and 1929. The results of the studies through 1928 were reported by Mirov (1), who conducted the 1928 tests, and the 1929 tests until August 20, when he accepted a position with the Forest Service.

It was at first believed that the volatile oils of the outer bark or of the oleoresin might contain the substance that determined the attractiveness of selected trees, and the 1928 studies emphasized this view of the problem. It was found, however, that these oils varied from neutral to decidedly repellent, and that the inner living bark, in which most of the carbohydrates are transported and stored, was apparently the most attractive part of the tree. It was also found that fermented inner bark was quite attractive. Since the inner bark is the part of the tree that is most important in the development of the brood, and since the contents and the condition of the inner bark would be directly affected by drought, defoliation and other conditions that make trees more attractive to the western pine beetle, it seemed logical to direct further studies toward a better understanding of this part of the tree.

The attractiveness of fermented inner bark also suggested the possibility of sugars and by-products of their fermentation as sources of the attractive substance. The chemical part of the study is separately reported on by Jeffrey (2), who determined the concentration of certain sugars in the inner bark of western yellow pine and how the concentrations varied under different conditions.

The 1929 attraction tests which are discussed in this report were planned to check the results of the 1928 tests, especially to determine more definitely the most attractive part of the tree, the comparative attractiveness of fresh samples from slow- and fast-growing trees, and the effect of fermentation on the attractiveness of inner bark and certain sugars. Tests were also run to study the change in attractiveness after a tree is felled and the possible repellent effect of turpentine and bark oils distilled from western yellow pine.

The field work was carried on at the North Warners field laboratory of the Bureau of Entomology between May 15 and October 15. A weather station was established and a continuous record of temperature and humidity was obtained by means of a hygrothermograph. The records are summarized in Plate 1.

New Equipment

Because of the time required for each test and owing to the intermittent nature of the beetle supply only a limited number of tests could be run in 1928 with the one set of equipment, consisting of rearing cage, testing cage and traps. In order to increase the number of tests that could be run, the following additions to our 1928 equipment were made for use in the 1929 tests:

1. A new 12x14-foot laboratory was built and equipped for sugar determination work, for the distillation of the volatile oils of the bark and for other laboratory needs in connection with the attraction tests.

2. A beetle-tight testing cage, 8 feet by eight feet by six feet in height, similar to the one used in 1928, was built near the new laboratory in a group of small pine trees.

3. A new rearing cage for collecting new adults as they emerged from the infested bark was also built near the new laboratory.

4. The fly-trap form of trap developed in 1928 was improved by removing the tin base and soldering short pieces of heavy wire to the bottom of the cylinder. This made the traps more accessible to the beetles, as the screen cone was raised only the thickness of the wire above the floor of the cage. Ten traps of this type were made, five being needed for each testing cage. (See Photo 1).

Preparation of Materials

No beetles were available for the attraction tests until June 20, because of the lateness of the season. In the meantime, from May 20 to June 20, Mirov prepared fermented inner bark[1], turpentine and bark oils for use in the tests. Large quantities of both inner and outer bark from selected trees of fast and slow growth were distilled and the oils collected.

[1]In these studies three different parts of the stem of the western yellow pine have been used--the sapwood, the inner bark and the outer bark. The term "inner bark" is used to designate the living tissues between the fascicular cambium on the inside and the phellogen or cork cambium on the outside. The fresh inner bark is a soft nearly white layer with high moisture content. It varies in thickness, depending chiefly on the growth rate of the tree, from 1/32 to $\frac{1}{4}$ inch. This is the layer in which the attacking parent adults make their egg galleries and lay their eggs, and in which the larvae feed until about one-quarter grown. The outer bark, as used here, is the dead cork layer, or the phellogen of the periderm. It is in this layer that the larvae complete their development, pupate and transform to the adult stage. Although the outer bark is almost impervious to moisture and air there is articulation between the inner bark and the outside by means of lenticels distributed throughout the periderm. These are made up of thin-walled cells separated by air spaces, so that an interchange of gases between the inner bark and the outer air is possible. Thus any unusual condition of the inner bark that resulted in the production of esters, aldehydes or other gases could be detected by beetles in flight.

In this distillation it was found that the outer bark from fast-growing trees contained up to twenty times the amount of volatile oil found in the outer bark of slow-growing trees. As this bark oil consists mostly of terpenes, which are believed to vary from neutral to repellent to the western pine beetle, the greater quantity found in the bark of the fast-growing trees may help to explain the preference for the bark from slow-growing trees which was evidenced by the beetles in some of the tests.

ATTRACTION TESTS

Methods

The procedure was in general similar to that followed in 1928. The *D. brevicomis* adults were collected as they emerged and congregated on the walls of the rearing cage, and released again in the testing cage (see Photo 2). The method of releasing the beetles was changed somewhat from the preceding year to insure a more even distribution throughout the cage. Instead of emptying the beetles on the floor, they were released on a small platform suspended in the center by wires from the top of the cage. The number of beetles introduced varied from 200 to as many as 1,000 in a day. It was not possible to determine the total number of beetles in the cage at one time, because they scattered around the cage and into cracks so that they could not be counted. The maximum number of active beetles in a cage at any one time was probably not over 2,000, with the average number nearer 1,000.

The arrangement of the traps was the same as that used last year--five traps to each testing cage, with four of the traps placed so as to form the corners of a square about four feet across, and the fifth trap in the center.

Considerable difficulty was encountered in an attempt to make the five trap positions equally attractive. Light, which varied throughout the day, was the most important factor, though temperature had also to be considered. In preliminary tests the same material was used in all five traps to determine the natural attractiveness of each trap position. It was not possible to make all the trap positions equally attractive, but the attractiveness of traps 1 and 2 (in Cage I) was made approximately equal to traps 3 and 4 by using 1x12 boards to shade the traps that received the most light. In the case of Cage II the northwest corner of the cage was much more attractive than the other positions, and shades, made by tacking heavy building paper over light wooden frames, were used to regulate the light. Although this decreased the difference in attractiveness of the various positions, it was found impossible to equalize them completely. Instead, a plan was worked out for comparing the different substances tested by using each substance in all the four outer traps.

This plan is as follows: In each attraction cage only two substances were tested at a time, two of the traps being baited with each substance. The fifth trap was used as a control. Each test ran for 48 hours and the substances were changed at the end of the first 24 hours, so that one substance was tested for the first 24 hours in traps 1 and 2 and for the second 24 hours in traps 3 and 4; while the other substance was tested in traps 3 and 4 for the first 24 hours and in traps 1 and 2 for the second 24 hours. The traps were emptied and reloaded with fresh material at 8:30 each morning, and the trapped beetles were collected and counted at 8:30 a.m. and at 1 and 5 p.m. Then by averaging the six counts for each substance--three from each pair of traps--we have a good basis for comparing the attractiveness of the two substances.

As this method of conducting the tests was not started till July 1, the few tests made in June did not have the light and temperature satisfactorily equalized.

Comparison of the Attractiveness of Inner Bark and Outer Bark

The logical starting point in this study seemed to be the determination of what part of the tree is most attractive to the western pine beetle. Then this part could be studied and analyzed further to determine the exact substance causing the attraction. The 1928 tests showed quite definitely that the bark, including both inner and outer layers, is more attractive than the other major parts of the tree, the wood and the needles. The same tests indicated a preference for the inner bark. The first studies of 1929 were planned to determine more definitely whether inner or outer bark is the more attractive.

Two series of tests were run for this part of the study, one in June and the other in the first part of July. The results are summarized in Chart 1.

In the first series of tests a total of 1,239 beetles were trapped, but the collections from the single control trap were doubled, so as to make the results comparable with those from the pairs of traps used with the bark. On this basis 640 beetles were trapped with the inner bark, 449 with the outer bark, and 300 (150 for the one trap) in the empty control trap. The analysis of the individual tests shows that in seven of the nine (in the first series) the beetles showed a preference for the inner, living bark; and in the two tests, 3-II and 18-II, in which the outer dead bark was preferred, the difference in favor of the latter was small. On a percentage basis, 46% of the total number of beetles collected were trapped with inner bark, 32.3% with outer bark, and 21.6% in the empty control trap.

The six tests made in July, in which light and temperature were more completely equalized, give about the same results. Of the 4,763 beetles trapped in this series, 2,159 or 45.3 per cent were trapped with inner bark, 1,608 or 33.8 per cent with outer bark, and 996 or 20.9 per cent in the empty control trap. In five of the six tests the beetles showed a preference for the inner bark; and in the other test, 26,28-II, the difference in favor of outer bark is very small, only 2.6 per cent.

It is noticeable that the number of beetles collected in the empty control traps is quite large--over 20 per cent of the total number trapped. Since there could be nothing attractive about an empty all-metal trap it may be assumed that their being trapped is purely accidental. Evidently the beetles crawl about on the floor of the cage more or less promiscuously, and many of them get caught accidentally in the control trap and in the baited traps as well. However, the fact that the number of beetles caught in the control trap was less than the number trapped with inner bark in every test, and greater than the number trapped with outer bark in only one test; and since more than twice as many beetles were caught per trap with inner bark as in the control trap, it is evident that bark, and especially inner bark, has a definite attraction for the beetles.

In the charts that have been prepared the attractiveness of the different substances tested has been compared on the basis of the total number of beetles trapped with each substance. If we assume, however, that a certain per cent of this total is the result of purely accidental trapping, and subtract this per cent from the total for each substance, we get a more accurate basis for comparison. Applying this to the results presented in Chart 1, for the six tests run in July, we would subtract, roughly, 20 per cent from the totals for both inner and outer bark. Then the actual ratio of attractiveness for inner bark and outer bark would be as 25.3 per cent is to 13.8 per cent. In the first method we find that outer bark is only 25 per cent less attractive than inner bark. By the second method, which seems more accurate, outer bark would be 45 per cent less attractive than inner bark, or only a little more than half as attractive.

In either case the evidence is sufficient to support the conclusion that inner bark is more attractive than outer bark, and so probably the most attractive major part of the tree.

It might be expected that the comparative attractiveness of inner and outer bark would vary with the growth rate of the tree. An examination of Chart 1 shows that this is apparently not true. Of the three tests in which outer bark was slightly more attractive than inner bark, slow-growing bark was used in two and fast-growing bark in one of the tests.

Comparative Attractiveness of Slow- and Fast-Growing Trees[2]

Earlier studies indicated a slightly stronger attraction of the western pine beetle to the bark of slow-growing trees than to the bark of fast-growing trees. This preference was not very definite, and a number of tests were run in 1929 to check this point.

Inner bark, outer bark and blocks--including inner bark, outer bark and sapwood--were taken from living trees of different growth rate for the tests. One test was made in June with light and temperature only partially equalized, and four tests were made in July with these factors more completely equalized. The results are given in Chart 2.

It is evident that there is no consistent preference shown for sections from either slow- or fast-growing trees. The most definite preference is shown in the case of the dead outer bark. In Test II-20 the outer bark from a fast- and from a slow-growing tree was compared. A preference was shown for the bark from the slow-growing tree, with which 102 beetles, or 45.3 per cent of the beetles trapped, were attracted, as compared with 69 beetles, or 30.7 per cent, with the bark of the fast-growing tree and 54, or 24.0 per cent, with the empty control trap. If we assume that 24 per cent of the beetles taken in each pair of traps was accidental, this leaves 21.3 per cent attracted to the slow bark and 6.7 per cent to the fast bark. Apparently the fast bark was almost neutral in its attractiveness, since only a few more beetles were trapped with the fast bark than with the empty trap.

It may be significant that the fast bark appears to be about as attractive as the bark oils which are distilled from it. See Chart 7. Bark oils are believed to vary from almost neutral to positively repellent, and the much greater quantity found in the fast bark than in the slow bark may account for the lack of attractiveness of the fast bark.

Only one test was run in 1929 for comparing fast and slow outer bark, but since the results agree with the 1928 tests it is probably true that the outer bark of slow-growing trees is more attractive than that of fast-growing trees.

Tests 34-40 inclusive (Chart 2) should be considered as four pairs of tests. As the beetles were more active on the first day of each test than on the second day, it was necessary to average the percentages trapped on the two days rather than the number of beetles.

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[2] It should be emphasized that this is not a comparison of the attractiveness of living trees of different growth rates, but of freshly-killed tissues from living trees. Caged-tree experiments and sample-plot studies with living trees have demonstrated a preference by the beetles for the slower-growing trees. This is evidently not true of samples removed from living trees.

The results indicate a preference for the fast inner bark in Tests 34 and 36, with 50.3% of the beetles trapped with the fast bark and 37.2% with the slow bark. Only 12.5% were trapped in the control trap. In tests 33 and 35 the slow-growing bark appeared to be more attractive when 44.8% were collected with the slow inner bark and 40.0% trapped with the fast bark. Only 15.2% were trapped in the control. The results are so contradictory that we can only conclude that no significant preference is indicated, though the fast inner bark is apparently more attractive in these tests.

The results of the comparison of green blocks, including inner and outer bark and sapwood, from fast- and slow-growing trees are just as inconsistent (Chart 2). In Tests 38 and 40 very little preference is shown, with 45.4% for the slow blocks and 46.0% for the fast. Only 8.6% was collected in the control traps. Tests 37 and 39 indicate a preference for the fast blocks, with 55.5% trapped compared with 34.5 for the slow blocks. Only 10.0% were trapped in the control trap.

The above tests taken as a whole show that there is no definite selection of freshly-cut parts of living, slow-growing trees, such as has been found to be true in the case of the living trees themselves. Evidently, as soon as a tree is felled or a part cut out a change takes place almost immediately that affects its attractiveness to the western pine beetle. Since the outer bark is already dead it would not be affected by being cut off, so it would continue to be more attractive on the slow-growing felled trees.

The outer bark appears to be more attractive on slow-growing trees, but the inner bark of fast-growing trees seems more attractive than the inner bark of slow-growing trees.

These tests also support the results of the first series of tests, which showed that the inner bark is more attractive than the outer bark. This is evident from the high percentage of beetles caught in the control traps when outer bark was being tested, and the low percentage caught in the control traps when inner bark was being tested. Outer bark from fast-growing trees was very little more attractive than the control trap, whereas inner bark from either fast- or slow-growing trees attracted nearly three times as many beetles as the control trap (Chart 2).

Changes in Attractiveness of a Tree After Being Felled

The original purpose of this series of tests was to compare the attractiveness of standing trees with trees which had been felled for different periods of time. The rapid change in attractiveness of samples after being taken from living trees made this impossible, so the tests were continued to obtain a comparison of the attractiveness of freshly-cut samples from living trees with the attractiveness of trees which had been felled for varying periods.

Blocks from Living Trees Compared with Blocks from Trees Which Had Been Felled for Three Weeks

In the first tests, numbered 41 to 48 inclusive, freshly-cut blocks of sapwood, with bark attached, from living trees were compared with similar blocks from short logs from trees that had been felled about three weeks before. In each test the standing tree and the felled tree used were of about the same rate of growth.

The results are presented in Chart 3. In all four pairs of tests the fresh blocks from living trees are more attractive to the beetles. Tests 41-44 inclusive compare the fresh and three-weeks-old blocks from slow-growing trees. In Tests 41 and 43, 303 beetles, or 56.2% of the beetles collected, were trapped with the blocks from the living tree; 192 beetles, or 35.6%, were trapped with the old blocks, and 44 beetles or 8.2% were trapped in the control traps. Tests 42 and 44 give a total of 513 beetles or 61.5% for the fresh blocks, 171 beetles or 20.5% for the old blocks, and 150 beetles or 18% for the control traps.

Tests 45-48 inclusive compare the attractiveness of fresh and three-weeks-old blocks from fast-growing trees. Tests 45 and 47 give a total of 130 beetles or 48.2% for the fresh blocks and 120 beetles or 44.4% for the old blocks. Only 20 beetles, 7.4%, were trapped in the control traps. Tests 46 and 48 give a total of 206 beetles or 57.8% for the fresh blocks and 126 beetles of 35.4% for the old blocks. Only 24 beetles, 6.8%, were collected in the empty control trap.

The results of the four pairs of tests give a total of 1,152 beetles or 57.6% trapped with the fresh blocks, and 609 beetles or 34.5% trapped with the three-weeks-old blocks. Only 11.9% of the total were trapped in the control traps. Assuming that about 12% is accidental trapping, we find that 45.6% were attracted to the fresh blocks and 22.5% were attracted to the three-weeks-old blocks. Thus the fresh blocks are apparently twice as attractive as the old blocks.

The small percent, 11.9, trapped in the empty control trap shows that both the fresh and the old blocks were definitely attractive to the beetles.

Progressive Changes in the Attractiveness of a Felled Tree

This series of tests was run to determine the progressive changes in attractiveness of the inner bark of a tree after being felled. This change was determined by comparison with a standing tree of the same size, vigor etc. Two fast-growing blackjack-type yellow pines about 16 inches diameter were selected for these tests. The trees were on the same site and as nearly alike in size, rate of growth, bark thickness and crown condition as we were able to find. Inner bark only was used for the comparison. Strips approximately three inches in width and six inches in length were used in each loaded trap. The strips averaged about 3/16 inch in thickness.

Tests were run for two days before either tree was felled to determine whether the inner bark of the two trees was naturally equally attractive. Then one of the trees, designated Tree No. 1, was felled, and the attractiveness of inner bark samples from the standing tree and the felled tree was compared for 16 consecutive days.

The results of these tests are tabulated in Chart 4 and shown graphically on Plate 2. The first tests, before either tree was felled, showed that the two trees were naturally about equally attractive, 50.8% of the beetles being trapped with Tree No. 1 (the tree that was felled)later) and 49.2% trapped with Tree No. 2 (the tree that was left standing). It should be noted that in this series of tests the control trap, #5, was used for a separate test which will be discussed under the next section, so thatthe beetles trapped in the four traps used to compare the standing and felled trees were figured as 100%.

After Tree No. 1 was felled the inner bark increased in attractiveness--as compared with the inner bark of Tree No. 2, which remained standing--for four days and then decreased in attractiveness during the remainder of the tests. The inner bark of the felled tree attracted 50.8% of the beetles at the time it was felled, 55.8% on the third and fourth days after being felled, and then decreased in attractiveness until only 38.9% were attracted on the fifteenth and sixteenth days after being felled. Although the differences in attractiveness of the two trees are not very great, there is a regularity about the curve (Fig. 2) that makes the results seem significant.

Apparently the disruption of the normal processes of the tree which follow almost immediately after the tree is felled or a sample removed, results in the strongest attraction, and a tree that has been felled for a week or more is less attractive than a freshly-killed tree or a fresh sample from a living tree. This agrees with the results of the first series of tests in this section, in which it was found that the blocks from living trees were more attractive than blocks from a tree that had been felled for three weeks.

Since the changes are probably mostly chemical, the rate of change in the attractiveness of felled trees would depend principally on the temperature.

Attractiveness of Fermented Inner Bark and Pure Sugars

The 1928 studies indicated that fermented inner bark is quite attractive to D. brevicomis, and in 1929 a number of tests were run to determine not only whether the fermented inner bark was generally attractive but also how this attractiveness varied with the type of fermentation, wild or controlled, and with the duration of the fermentation. The attractiveness of pure yeast cultures in dextrose and maltose media were also tested.

Relation of Duration of Natural Fermentation to Attractiveness

A number of different tests were grouped so as to bring out this relation, of the length of time that the inner bark had been fermenting to its attractiveness. In tests numbered 52-54, 56-58, 60-61, 62-64, 69-71 and 73 the fermented inner bark was prepared by filling a gallon jar with strips of inner bark and covering them with water. The jar was left uncovered, the inner bark soon became infected with wild yeast from the air, and fermentation began within a short time. The attractiveness of this fermenting inner bark was compared daily with that of fresh inner bark from a living tree for eight days after fermentation began.

The results are summarized in Chart 5 and shown graphically on Plate 3. Evidently the fermenting inner bark was most attractive just after fermentation began, and decreased in attractiveness as fermentation progressed. The one-day-old fermented inner bark attracted 58% of the beetles, compared with 42% for the fresh inner bark. The eight-day-old fermented inner bark attracted 46.8% of the beetles, compared with 53.2% for the fresh inner bark. The attractiveness of the fermented inner bark decreases consistently from 58% of the total on the first day to 55.2% on the second, 51.8% on the third and fourth, 49.8% on the fifth and sixth and 46.8% between the seventh and eighth. In this comparison 50% would represent equal attraction to both fermented and fresh inner bark.

Although the differences in attractiveness of fresh and fermented bark are not great at any time, there is such a consistent decrease in the attractiveness of fermented bark that the results are probably significant. It is reasonable to expect that as fermentation progresses certain repellent products might be formed which would reduce the attractiveness of the fermentation. This would be especially true in this case, where the inner bark was exposed to contamination from the air.

Pure Yeast-Inoculated Inner Bark Compared with Moistened Cotton

This series of tests, made in connection with tests to determine the changes in the attractiveness of a tree after being felled (discussed in the preceding section), gives definite proof of the attractiveness of inner bark inoculated with a pure yeast culture. The material used was prepared as in the preceding fermentation studies, except that contamination from the air was prevented as far as possible and the inner bark strips in water were inoculated with a concentrated pure yeast culture in a dextrose medium. This yeast is the one commonly found on the western pine beetle and in its alimentary tract.

Only one trap, the fifth trap ordinarily used for the control, was used in these tests. On one day it was loaded with fermenting inner bark and on the next day with cotton moistened with water to equalize, at least partially, the effect of differences in moisture content. The position of the trap was not changed. Strips of the fermented inner bark were used on the eighth, ninth, eleventh, thirteenth, 15th, 17th, 20th and 22nd days after fermentation had started. The moistened cotton was used on the other days--the 7th, 10th, 12th, 14th, 16th and 21st.

The results are tabulated in Chart 6 and shown graphically on Plate 4. It is evident that fermenting inner bark is decidedly more attractive than moistened cotton. Averaging the percentages trapped with fermenting inner bark and for moistened cotton separately, we find that 19.46% of the beetles trapped in these tests were trapped with fermenting bark and only 10.6% with moistened cotton. In both cases the percentages appear low because only one of the five traps was used for this study. Since in nearly every instance the number of beetles trapped practically doubles when the substance is changed from moist cotton to fermenting inner bark, and is reduced by nearly one-half when changed back to moist cotton, we may conclude that pure yeast-inoculated inner bark is definitely attractive to the beetles.

The results of this study may help to explain the increase in attractiveness of trees after a few attacks have been established. It was found in our caged-tree studies that a moderately attractive tree became highly attractive after a few attacks were established by caged beetles. This increase in attractiveness may be due to a fermentation which is started by the yeasts introduced by the attacking beetles.

Attractiveness of Fermented Inner Bark and Sugar Solutions
Miscellaneous Tests

There are included in this section a number of unrelated tests which must be considered individually. They are included principally as a matter of record, so that if other similar tests are made they may be compared. The results are tabulated in Chart 7.

Test 50-51 compares the attractiveness of fresh inner bark with that of fermented bark extract on cotton. The inner bark extract had been fermenting for about two months. There is no significant difference in the attractiveness of the two materials, fresh and fermented.

Test 53-55 compares two-months-old fermented bark extract on cotton with fresh water on cotton. The old fermented bark extract is evidently less attractive than water-moistened cotton.

The next three sets of tests, 57-59, 66-68 and 70-72, compare the attractiveness of four-months-old pure yeast cultures in different sugar solutions--sucrose, dextrose and maltose--with newly-fermented inner bark and fresh inner bark. In all these tests a definite preference is shown for the inner bark, either fresh or fermented. Evidently the yeast produces attractive substances only when growing on inner bark of western yellow pine.

Test 63-65 compares the attractiveness of fast- and slow-growing inner bark, both of which had been fermenting for seven days. No significant preference is shown. This agrees with other data and observations, and supports the belief that although slow-growing trees are more attractive than fast-growing trees so long as both are living, after they are felled one is no more attractive than the other.

Test 104 shows, in agreement with other tests, a preference for inner bark inoculated with pure yeast when compared with fresh inner bark.

Turpentine and Bark Oils as Possible Repellents

Although no definite series of tests were run to study repellents, turpentine and bark oil, which have been considered as repellents, were used in connection with other tests with fresh and fermented inner bark.

The turpentine was distilled from the oleoresin and the bark oil from the bark of western yellow pine. The percentage of beetles trapped with the oil and turpentine was compared with the percentage taken in the empty traps or with moist cotton. The results are summarized in Chart 8.

In tests 74 and 75 the attractiveness of turpentine was compared with that of fresh inner bark and with the empty control trap. Two traps of the five were used for the turpentine, two for the inner bark, and the fifth was left empty for a control. The collections from the control trap were multiplied by two to make them comparable with the collections from the other two pairs of traps. The results indicate that the turpentine is repellent, since only 17% of the beetles were trapped with the turpentine compared with 25.3% in the empty trap. However, these results are not as reliable as the other tests in this section because the "position attractiveness" of any pair of the four outer traps has never been equalized with that of the center, or fifth trap. In the preliminary tests, in which the same materials were used in all five traps, the fifth trap attracted on the average about as many beetles as the other traps; but the percentage varied in the different tests, so that the results from any one test would not be of great value.

In the other tests included in this section turpentine was used in the check trap in tests 68-71 inclusive, bark oil was used in tests 66 and 67, and in tests 72 and 73 the check trap was left empty. All these tests were run at about the same time, between August 14 and 18, and under the same conditions, so the results should be comparable. The results (Chart 8) show very little difference in the reaction of the beetles to turpentine and bark oil. They also indicate the lack of either an attractive or a repellent character. What little effect is shown is in the direction of attractiveness; for whereas 12.6% of the beetles collected in these tests were trapped in the empty trap, 13.3% were trapped with turpentine and 15.4% with bark oil.

Because of the small number of tests and the difference in results, no definite conclusions can be drawn. It seems evident, however, that neither turpentine nor bark oil is either attractive or repellent to any marked degree.

GENERAL DISCUSSION

Most of the discussion was included under the appropriate section in connection with the statement of results. There are a number of points, however, which apply to the project as a whole rather than to any one section, and these will be considered here.

Theory of Tree Selection by the Western Pine Beetle

As a result of our attraction studies, including the chemical phases treated by Jeffrey, supplemented by general observation in connection with the use of trap trees and general control work, a tentative theory has been developed in an attempt to explain how it is possible for the western pine beetle to select certain trees in a yellow pine stand. This theory is as follows: The attraction that results in the complete attack of a tree includes two stages--the initial stage of attraction, resulting from a respiratory fermentation induced by some condition unfavorable to the normal growth of the tree, and a later stage of much more definite attraction following the development of yeasts and other microörganisms which are introduced by the first beetles making successful attacks.

It is assumed that the western pine beetle is definitely chemotropic, and is affected by odors of such a nature and in such quantities that they could not be detected by man. In view of the recent work that has been done on chemotropism among insects by Minnich, McIndoo and others, as well as our own studies, this assumption does not seem unreasonable. The fermentation of the inner bark by yeasts introduced by the first beetles to attack and the attractiveness of this fermenting inner bark have already been pretty well worked out.

The most difficult part of the theory is in regard to the physiology of the initial respiratory fermentation which, to the writer, appears to be the heart of the whole tree-selection problem. Apparently some change takes place in the inner bark of felled trees, fire-injured trees, top-killed trees and trees suffering from a deficiency of moisture that makes them more attractive to the western pine beetle. The most logical explanation seems to be that a type of respiratory fermentation takes place, a by-product of which is some aldehyde, ester or similar substance that is particularly attractive to the beetles. This type of fermentation is recognized by plant physiologists, who explain it as a result of the disorganization of the normal plant processes and particularly the abnormal activity of certain plant enzymes.

Our attraction studies, as well as our work with trap trees, support the belief that while the change in a standing tree may take place gradually in felled trees or in parts removed from living trees, the change from unattractive to attractive takes place within a very short space of time. This initial change did not result in any noticeable change in the sugar concentrations, and it is doubtful if this change can be demonstrated chemically, because it probably takes place while the sample is being prepared for analysis.

The most significant result of the sugar analyses made by Jeffrey is the relation between the sugar concentrations of trees of extremely slow growth and trees after being felled. It was found that the slower the growth rate of the tree the higher the percentage of levulose and the lower the percentage of sucrose; and that after a tree is felled the concentration of levulose increases and the concentration of sucrose decreases. This difference evidently appears before the felled tree is attacked, but increases after attack. This indicates that the change occurring in both stages of attraction is similar, but that it is greatly accelerated by the yeasts introduced by the attacking beetles.

Variability in the Response of the Beetles

It is very evident from the data presented that none of the substances tested could be used with any degree of success as an artificial attrahent. Even with the most attractive substance tested, fermented inner bark, only a limited number of beetles were trapped, and from 4 to 24% of the beetles trapped in any one experiment were trapped in the empty control trap. This same variability in the response of insects to attrahents has been found in practically all the work that has been done with cutworm moths, codling moths, potato beetles--and even in the case of the Japanese beetle there is evidently little probability of the geraniol traps being of much value except in biological and distributional studies.

In all the work that has been done with the western pine beetle this lack of uniformity in the response of the beetles has been noticeable. This was found true in the case of laboratory experiments and it is also true in the field. Trap trees are usually attacked much more readily than uninjured standing trees, and yet it was found impossible to absorb all the infestation of an area with trap trees, regardless of the number and distribution; and beetles emerging from a pile of bark within a few feet of highly susceptible trees which were just being attacked would fly off in another direction. Other examples could be cited, all of which support the belief that all D. brevicomis adults cannot be attracted by any one substance, and also that the same beetles will react differently at different times. It is very doubtful, therefore, whether it will be possible to find any substance attractive enough to be of value in trapping beetles as a control measure.

In spite of this variability in the reactions of beetles, the results of our tests are quite consistent, in that different tests or series of tests with the same materials agree very well in the difference in attractiveness of materials as shown by the percentage of beetles attracted to each. This is evident in Chart 1, where the tests made in July agree so completely with the June tests of the same materials; in Figs. 2 and 3, where the curves showing the changes in attractiveness of felled trees and fermenting inner bark are remarkably smooth; and in Fig. 4, where the attractiveness of fermented inner bark is consistently nearly twice as attractive as the moist cotton that was used as a check.

It seems probable that of the beetles being tested at any one time, some are much more responsive than others, and it is these beetles that show the relative attractiveness of different materials. Likewise, in the field, certain more sensitive beetles are attracted to the subnormal trees, where they initiate the attack that is continued and made successful by the less sensitive individuals.

SUGGESTIONS FOR FURTHER STUDIES

In a study of this kind new fields are continually being opened up, and it is difficult to confine the work to leads that give reasonable promise of practical application. After two years' work it seems desirable to decide along what lines this study should be continued, and how far we are justified in going with a study of this kind.

A theoretical explanation for the tree selection habits of the western pine beetle has been worked out, and it is suggested that further work should be directed toward proving or disproving any part of this theory that is susceptible of proof on the basis of our present knowledge. According to our present theory, the attractive substance itself is some aldehyde, ester or similar substance, produced in such small quantities and such complicated mixtures that there is very little hope of isolating, identifying or testing it. No work should be attempted on this part of the problem. However, an index to the production of this attractive substance may be found in the changes in sugar concentrations that accompany it. Jeffrey found that felled trees and trees of slow growth rate were similar, in that the levulose content was higher and the sucrose content (of the inner bark) lower than in normal, healthy trees. This work should be continued, first to check the results of the 1929 determinations, and second to extend this work to find whether this sugar relation holds true for other trees known to be attractive to the western pine beetle, especially fire-injured and top-killed trees.

Jeffrey also found that the pH value and the moisture content of slow-growing trees was lower than for fast-growing trees. Work on these two factors should be extended in line with the work on the sugar concentrations. Since Beal and Keen are doing some work on moisture relations it would probably be best to extend our work more on the relation of pH values than on moisture. Then too, although it has been known that drought is probably the most important factor in increasing the number of attractive and susceptible trees, it is hard to understand how a slight difference in the moisture of a tree, with no accompanying chemical change, could make it more or less attractive to the beetles. The chemotropic response of the beetles is fairly well established, whereas little or nothing is known concerning their reaction to differences in moisture.

As to the testing of different substances, it is suggested that most of our work in the future be along the following lines: The testing cages as used last year might be utilized for testing pure chemicals of supposed attractive or repellent qualities, something that has not yet been done; but otherwise little work should be done along this line. Further work with traps should be similar to the Japanese beetle work, where the substances are tested in the field on beetles in flight. Probably the only way of comparing the attractiveness of two trees while still living is by caging them together in one trap and then introducing beetles and checking their reactions to the two trees. If trees suitably located can be found, the effects of top-pruning, fire injury, girdling, root-pruning and other types of injury on the attractiveness of the trees can be determined by comparison with normal trees with which they would be caged.

LITERATURE

(1) Mirov, N.T. - A Preliminary Study of Attraction with the Western Pine Beetle, Dendroctonus brevicomis Lec.; Ms. Dec. 5, 1928

(2) Jeffrey, R.N. - The Concentration of Certain Sugars in the Bark of Western Yellow Pine as Related to Western Pine Beetle Attraction; unpublished report, Feb. 1930

C H A R T 1

Comparative Attractiveness of Inner and Outer Bark

	Light and Temperature Effects Not Equalized										Light and Temperature Effects Equalized						
Cage No.	II	I	II	I	II	II	I	II	I	Totals 9	I	I	I	II	II	II	Totals 6
Test No.	1-II	2-I	3-II	4-I	5-II	16-II	17-I	18-II	19-I	Tests	21-23	25-27	29-31	22-24	26-28	30-32	Tests
Date	6/20	6/21	6/21	6/22-23	6/22	6/28	6/29	6/29	6/30	6/20-30	7/1-2	7/3-4	7/5-6	7/1-2	7/3-4	7/5-6	7/1-6
	Inner and Outer Bark from Living Trees								From log felled two wks.		Inner & Outer Bark fm. Living Trees						
											Med.	Slow	Fast	Med.	Slow	Fast	
Slow	1	21	5	8	18							324			430		
Inner Bark	3	30	18	50	62	89	209	160	51	640	393	324	285	402	430	327	2159
Fast	2	9	13	42	44	89		160					283			327	
Slow	0	3	17	9	34							187			447		
Outer Bark	0	6	24	39	39	50	102	167	34	449	180	187	154	325	447	315	1608
Fast	0	3	7	30	5	50		167					154			315	
Control (X2)	0	0	4	14	10	18	130	104	20	300	160	112	136	178	276	134	996
Total D.b. trapped										1389	Total D.b. trapped						4763
% of Total No. of Tests Showing Preference for Inner Bark										78.0							83.5
" Outer "										22.0							16.5
% of Total No. of D.b. Trapped with Inner "										46.0							45.3
" Outer "										32.3							33.8
Control										21.6							20.9

CHART 2

Comparative Attractiveness of Slow and Fast Growing Trees

Cage No.	II		II	II	Av.%	I	I	Av.	II	II	Av.%	I	I	Av.%
Test No.	20	%	34	36	34-36	33	35		38	40		37	39	
Date	6/30		7/7	7/8		7/7	7/8		7/9	7/10		7/9	7/10	
Explanation	Out. Bark		Inner Bark			Inner Bark			Green Blocks Sapwood and Bark			Green Blocks Sapwood and Bark		
Slow Growth	102	45.3	348-63.5	19-10.9	37.2	116-47.2	66-42.3	44.8	48-25.0	133-65.8	45.4	134-33.0	108-36.0	34.5
Fast Growth	69	30.7	120-21.9	137-78.8	50.3	96-39.0	64-41.0	40.0	134-69.8	45-22.2	46.0	224-55.0	168-56.0	55.5
Control (X2)	54	24.0	80-14.6	18-10.3	12.5	34-13.8	26-16.7	15.2	10- 5.2	24-11.8	8.6	48-12.0	24- 8.0	10.0
Totals	225					246	156	100.				406	300	100.0

CHART 3

Comparative Attractiveness of Old and Newly-Felled Trees

									Summary	
Cage No.	1		2		1		2		1 & 2	
Test No.	41.43		42.44		45.47		46.48		41 - 48	
Date - July	11,12		11,12		13,14		13,14		11 - 14	
Explanation	Slow-Growing Trees				Fast-Growing Trees				Fast & Slow	
		%		%		%		%		%
Freshly-Cut Blocks (Sapwood with bark attached)	303	56.2	513	61.5	130	48.2	206	57.8	1152	57.6
Block from 3-weeks-old logs	192	35.6	171	20.5	120	44.4	126	35.4	609	34.5
Control (X 2)	44	8.2	150	18.0	20	7.4	24	6.8	238	11.9
Total number of D.b. collected	539	100.	834	100.0	270	100.	356	100.	1999	100.0

C H A R T 4

Change in Attractiveness of Felled Tree

	Both Standing	No. Days After Tree No.1 Was Felled							
		1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16
Experiment No. 6	A & B								
Date	3,4	5,6	7,8	9-10	11-12	13-14	15-16	17-18	19-20
Tree No. 1 Felled Sept. 4	188 50.8%	163 52.9	192 55.8	242 50.2	298 44.6	206 42.5	173 46.1	101 56.7	49 38.9
Tree No. 2 Left Standing	183	145	152	240	370	278	202	174	77
Totals	371	308	344	482	668	484	375	275	126

C H A R T 5

Attractiveness of Fermented Inner Bark Compared with Fresh Inner Bark

Natural, Wild Yeast Inoculation

Exp. No.	69-71		52-54		73		56-58		60-61		62-64	
Cage No.	II		II		II		II		II		I	
Date--August	15-16		6-7		17		8-9		10-11		12-13	
Explanation Age of Fer-ment in Days	1		1-2		2		3-4		5-6		7-8	
	No.Db	%	No.Db	%	No.Db	%	No.D.b.	%	No.D.B.	%	No.D.b.	%
Fermented Inner Bark	739	58.0	253	57.4	274	55.2	450	51.8	509	49.8	120	46.8
Fresh Inner Bark	537	42.0	188	42.6	222	44.8	419	48.2	513	50.2	136	53.2
Total D.b. Attr. to Inner Bark	1276		441		496		869		1022		256	
Control (empty)			102	18.8	104	17.3	200	18.7	208	16.9	36	12.3
Turpentine	192	13.0										

C H A R T 6

Attraction of D.brevicomis to Fermented Phloem
Compared with Water-Moistened Cotton

Test 106

No.of Days After Inoculation	No. of Beetles Attracted in Per Cent Moist Cotton	Fermenting Phloem
7	9.25	
8		21.40
9		16.50
10	11.20	
11		18.90
12	8.57	
13		20.62
14	8.20	
15		16.00
16	11.58	
17		17.28
18		
19		
20		22.65
21	14.80	
22		22.30
Total Per cent.	63.60	155.65
Aver.	10.60	19.46

C H A R T 7

The Attractiveness of Fermented Phloem and Sugar Solutions

Miscellaneous Tests

Experiment No.	50.51		53.35		57.59		66.68		70.72		63.65		104a,b	
Cage No.	I		I		I		I		I		II		II	
Date - August	5,6		7,8		9,10		14,15		16,17		12,13		30,31	
Explanation	2 mo.old ferment vs. fr. inner bark		2 mo.old ferment vs. moist cotton		Old yeast in suc.vs.4-5 day fermented inn.bark		Old yeast in dex.vs.fresh inner bark		Old yeast in malt vs. fr. inner bark		Fast vs.slow 7-8-day fer. inner bark		Pure yeast on inner bark 4-5 days vs.fr. inner bark	
		per cent		per cent		per cent		per cent		per cent		per cent		per cent
Ferments														
I.b.ext.on cot.	161	41.0	115	36.5	244	51.6								
Slow in.bark											717	45.7	630	45.0
Fast " "											726	46.3		
Old Dex.sol.							90	35.0						
" Malt."									57	27.8				
" Sucr."					187	39.5								
Fresh In. Bark	154	39.2					123	47.8	130	63.4			468	33.4
Moist Cotton			144	45.7										
Control (empty)	78	19.8	56	17.8	42	8.9			8	3.9	126	8.0	302	21.6
Bark Oil							22	8.6						
Terp.							22	8.6	10	4.9				
Totals by Tests	393		315		473		257		205		1569		1400	

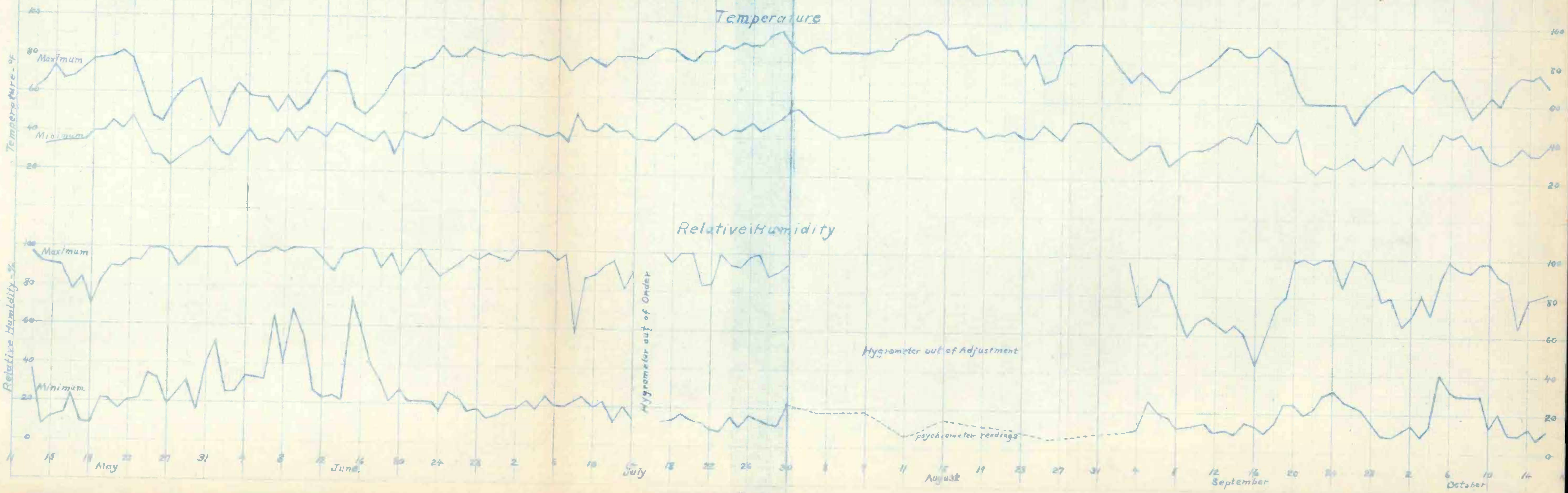
CHART 8

Turpentine and Bark Oils as Possible Repellents

Experiment No.	74	75			68	70	69	71			66	67		72	73	
Cage No.	II	II			I	I	II	II			I	II		I	II	
Date - August	19	20			15	16	15	16			14	14		17	18	
Explanation			Totals	%						Av.%			Av.%			
Fresh Inner Bark	139	238	377	57.2	77	49	249	288	663	39.0	46	243 288		81	222	
Fermented " "							368	371	810	47.7					274	
Sugars					25	46					65			11		
Turpentine	56	56	112	17.0	22	10	104	88	224							
% of Total					17.7	9.5	14.4	11.8	534	13.3						
Bark Oil											22	88				
% of Total											16.5	14.2	15.4			
Check, Empty(X2)	80	90	170	25.8										8	104	
% of Total														8.0	17.3	12.6
Total			659	100.0												

Plate I

Climatological Data



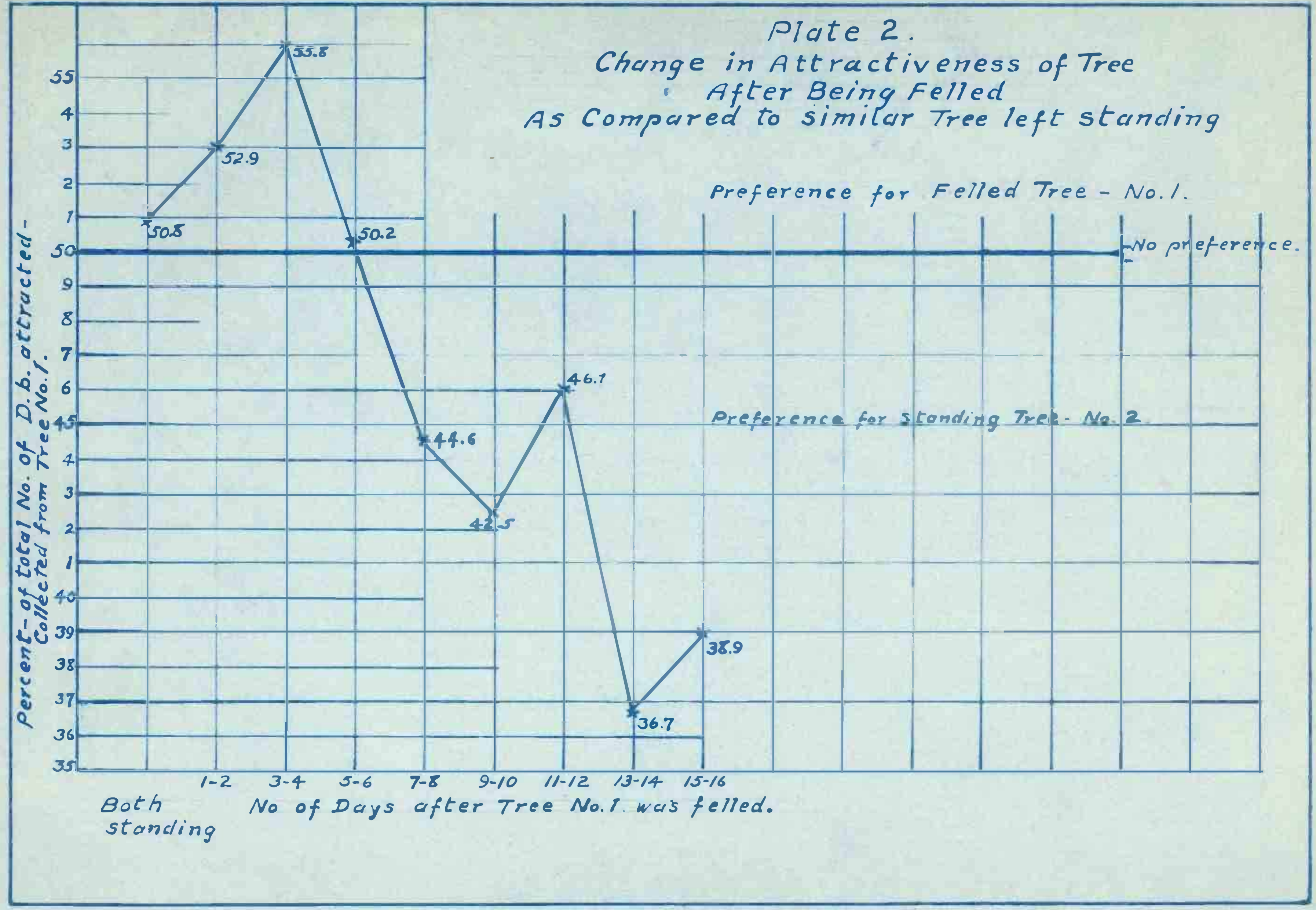
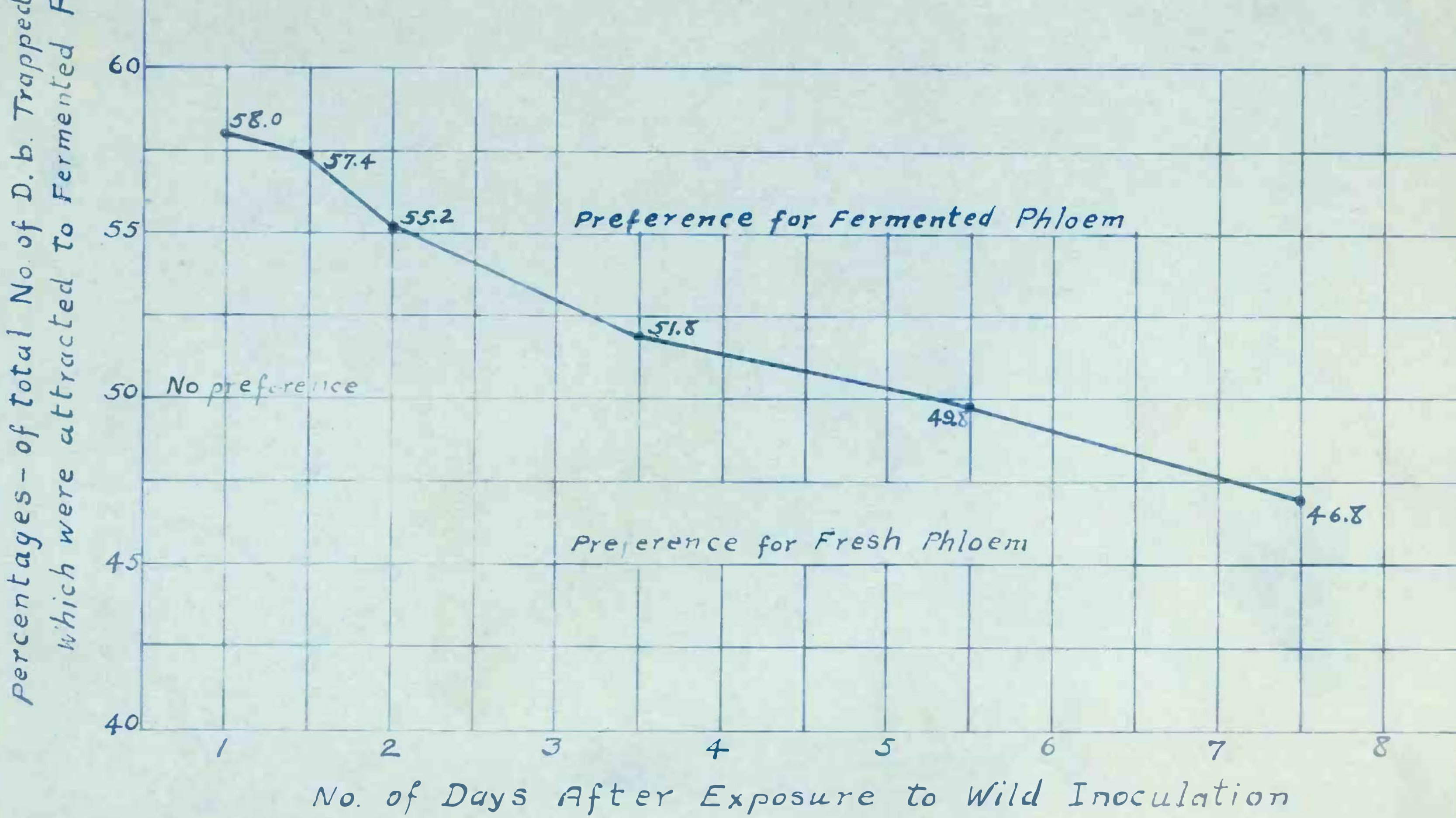


Plate 2.
Change in Attractiveness of Tree
After Being Felled
As Compared to similar Tree left standing
Preference for Felled Tree - No. 1.
No preference.
Preference for standing Tree - No. 2
Percent - of total No. of D. b. attracted - Collected from Tree No. 1.
50.8
52.9
55.8
50.2
44.6
42.5
46.1
36.7
38.9
55
4
3
2
1
50
9
8
7
6
45
4
3
2
1
40
39
38
37
36
35
1-2
3-4
5-6
7-8
9-10
11-12
13-14
15-16
Both standing
No of Days after Tree No. 1 was felled.

Plate 3.
Attraction of *D. brevicomis* to Fermented Inner Bark.
Compared with Fresh Inner Bark one to Eight Days after Inoculation



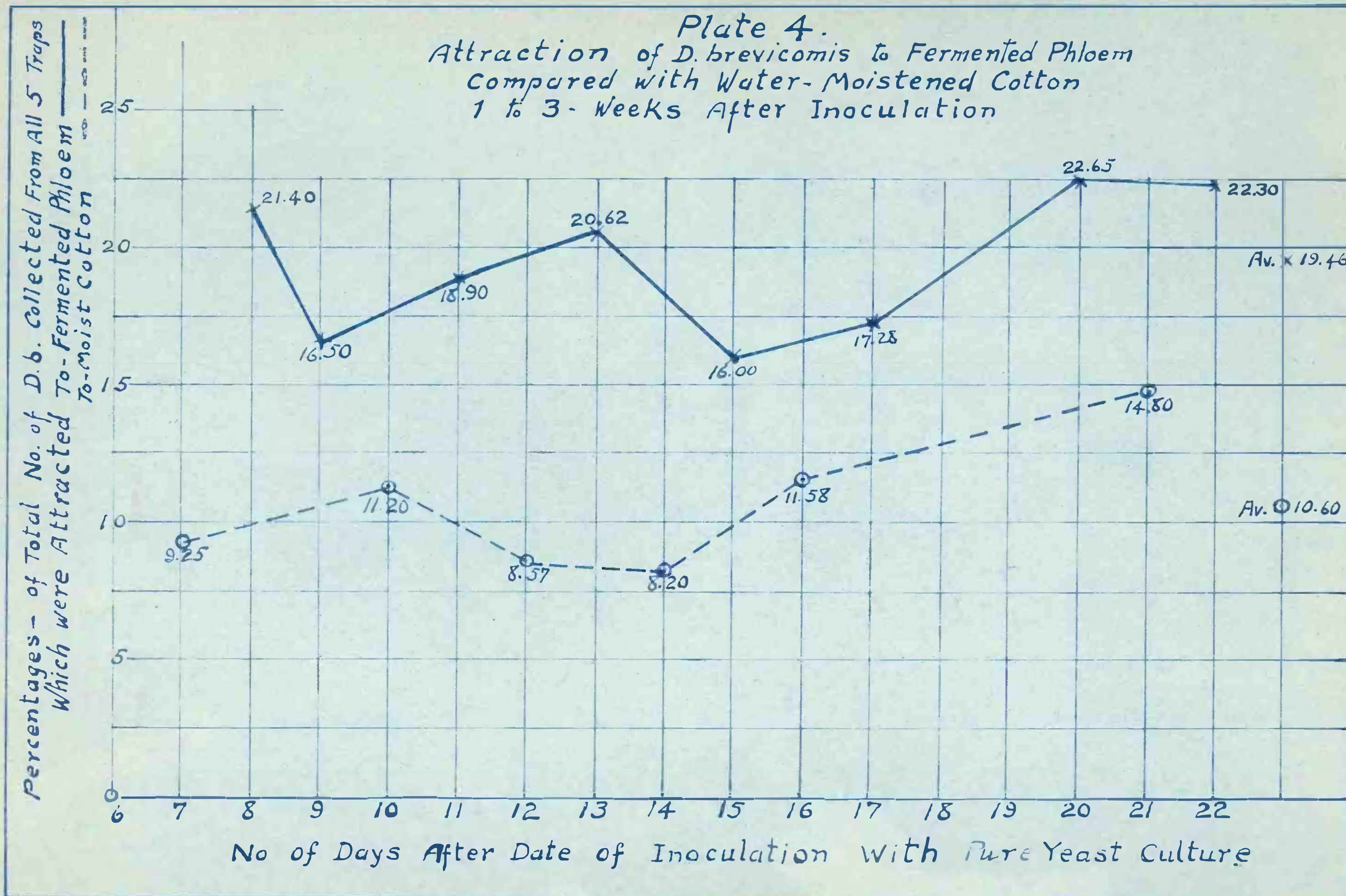


Plate 4.
Attraction of D. brevicomis to Fermented Phloem
Compared with Water-Moistened Cotton
1 to 3 - Weeks After Inoculation
Percentages - of Total No. of D. b. Collected From All 5 Traps
Which were Attracted To-Fermented Phloem
To-Moist Cotton
25
20
15
10
5
0
21.40
16.50
18.90
20.62
16.00
17.28
22.65
22.30
Av. × 19.46
9.25
11.20
8.57
8.20
11.58
14.80
Av. ⊙ 10.60
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
No of Days After Date of Inoculation With Pure Yeast Culture

-31-

Photo 1 - Cylinder type traps, showing arrangement of the "loaded" traps on the floor of the testing cage. Pieces of phloem, bark and sapwood may be seen in the traps. (Photo by Person)

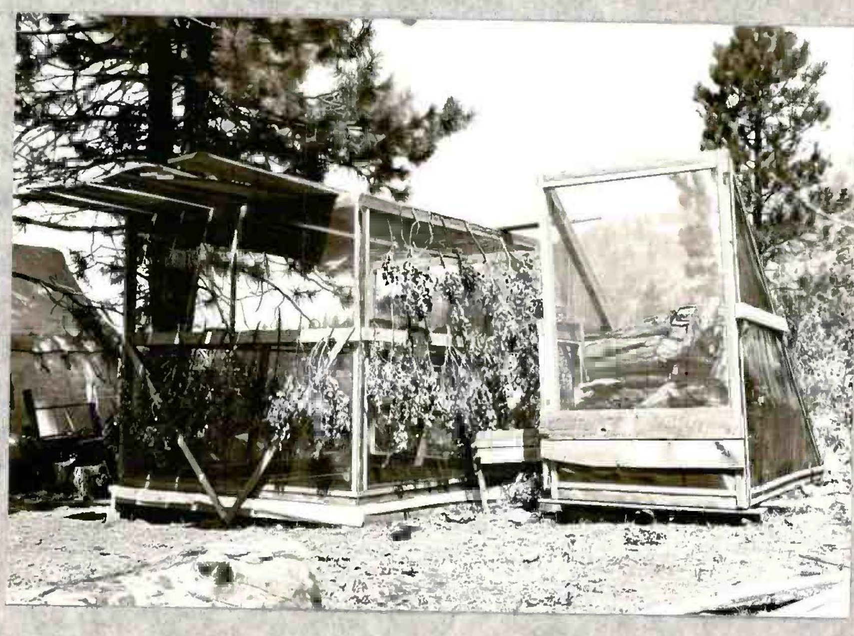


Photo 2 - Cages used in attraction studies. The <u>rearing cage</u> (on the right) was used to collect the beetles as they emerged from the infested bark. Beetles were collected from the front wall of the cage and transferred to the <u>testing cage</u> (on the left). Beetles were released in this cage and loaded traps placed on the floor to test the comparative attractiveness of the different substances (photo by Person).
This photo was taken in 1928. In 1929 boards and building-paper shades were used instead of brush for equalizing the light on the different traps.